



MEMORANDUM

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DATE: January 7, 2011
TO: Dan Graham, PacRim Coal, LP
FROM: Jerry Diamond and Henry Latimer
SUBJECT: Results of confirmatory WER mixture testing

Background

Based upon discussions between PacRim Coal, LP, Alaska Department of Environmental Conservation (ADEC), EPA, and Tetra Tech, it was determined that prior to adoption of site-specific criteria based on the water-effect ratio (WER) submitted by PacRim (March 2010) confirmatory testing of the proposed criteria were necessary. The goal of this testing is to confirm that the proposed site-specific criteria for aluminum (Al), copper (Cu), lead (Pb), and zinc (Zn) will not cause toxicity when tested together at the proposed criteria concentration for each metal.

This testing was conducted following the methodology outlined in our December 3, 2010 memo which was developed following additional discussions and comments from ADEC and EPA.

Methods

Testing was conducted using both *Daphnia magna* and *Pimephales promelas* because *P. promelas* was the primary species (i.e., more sensitive species) for aluminum while *D. magna* was the primary species for the other three metals. Attachment A summarizes test conditions used in this study. Because WER values were generated using acute toxicity testing methods, the acute site-specific criteria developed from the WER studies, were used to develop the solutions for testing. Although an acute site-specific aluminum criterion has not been proposed, due to the naturally low hardness of the Chuitna surface waters, the Alaska standard of 750 µg/L (a value much higher than the proposed site-specific chronic aluminum criteria), was used for these tests in order to satisfy concerns regarding potential toxic contributions from aluminum.

A grab sample was collected from Chuitna site 141, the same location where other site water samples were collected for WER testing. Samples were packaged and shipped to Tetra Tech's laboratory in Owings Mills, MD on December 7, 2010. This sample was handled and transported in the same manner as all other WER samples (as described in the August 19, 2009 Study Plan and in Tetra Tech's WER Report March 12, 2010). Two sub-samples of this sample were prepared for analysis of total and dissolved organic carbon and both total and dissolved metals (Al, Cu, Pb, and Zn). In addition to these analyses pH, conductivity, dissolved oxygen, alkalinity, and hardness were measured on the sample upon receipt in the laboratory. The sample to be analyzed for dissolved metals was field-filtered within 15 minutes of collection.

The site water sample could potentially have some metals naturally based on historic data as well as data collected in WER testing. Therefore, it was critical that site water for Al, Cu, Pb, and Zn be measured prior to testing so that the proper concentrations of each metal were used in mixture testing. To this end, the filtered metals, total metals, and other chemistry samples were transported to Microbac Laboratories in Baltimore, MD with a 24-hr turn-around time on the metals analyses. Because of the time required to ship and analyze the metal concentrations in these samples, testing was initiated approximately 96 hrs following collection. This holding time is believed to be inconsequential to the test results because site water was stored sealed at $< 4^{\circ}\text{C}$ during this time and therefore biological activity or other potential changes to the sample were unlikely.

Chuitna water was spiked with aluminum, copper, lead, and zinc to the total metal concentrations that were associated with dissolved metal criteria based on individual metal WERs. All four metals were added as highly soluble chloride salts ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, PbCl_2 , ZnCl_2 , and $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), consistent with WER testing and EPA recommendations. Note that these metals are very unlikely to occur or be discharged as soluble chloride salts because these metals typically do not occur as free ions in natural surface waters (hence the water-effect ratios observed). The metal salts used in testing readily disassociate into component ions upon introduction to water. This is demonstrated by the dissolved metal concentrations observed in lab water in single metal WER testing (Table 1). Lab water is required to have little or no metal-complexing ability due to the absence of organic carbon, solids, and chemical ligands. Thus, most of the metals in lab water are in a dissolved form due to the absence of complexing agents, similar to what EPA reported in the criteria for these metals (Table 1). However, given the natural organic carbon and ligands (e.g., TDS) observed in the Chuit site water, free metal ions rapidly combine with other compounds to form larger complexes that may not remain "dissolved" (where dissolved is defined as having the ability to pass through a $0.45\ \mu\text{m}$ filter). In relatively simple solutions (e.g., low to no organic matter, and few other complexing agents), much of the metal might be expected to remain in the dissolved form, as reflected in Tetra Tech's lab water and in EPA's dissolved to total ratios for these metals (Table 1). In the Chuit site water, much of the free ionic metal added is rapidly complexed and no longer available in a dissolved form in test chambers (Table 1). Thus, while nearly all of the total metal added to Chuit site water is in a dissolved form initially, it quickly (within one hour) is bound to natural constituents in the site water and is no longer measurable as dissolved. This is in fact the mechanism that is quantified using the WER procedure. This is especially the case for lead but is true for copper and zinc as well (Table 1).

Table 1. Summary of dissolved:total ratios for copper, lead, and zinc taken from EPA criteria, single-metal WER testing in both laboratory synthetic and Chuitna water, and mixture testing.

Metal	Dissolved:Total Ratio			
	From EPA Criteria	From single-metal WER testing in lab water	From single-metal WER testing in Chuitna water	From mixture testing
Cu	0.96	0.90	0.81	0.42
Pb	0.93	1.02	0.49	0.08
Zn	0.98	0.98	0.77	0.50

Chuitna water was spiked with aluminum, copper, lead, and zinc to the total metal concentrations listed in Tables 2 and 3. In addition, un-manipulated Chuitna water and reconstituted laboratory control water was also tested. All methods followed EPA protocols used in WER testing (USEPA 2002), however the number of *D. magna* per replicate was increased from 5 to 10 in each test chamber to obtain greater test power and statistical confidence in the results. Following test completion at 48h, organism survival in the spiked Chuitna sample and the laboratory control (laboratory synthetic water) was compared to that of the un-manipulated sample with a t-test. The results of the test were determined to be “acceptable” (i.e., the mixture of metals at the site-specific criterion concentrations do not cause significant effects) if there was not a significant decrease in the survival of the spiked sample relative to the survival in the un-manipulated sample (alpha level of 0.05).

Results

Data from metal mixture testing indicated that none of the three treatments resulted in significant (alpha = 0.05) mortality in the *D. magna* tests (Table 2). The spiked site water sample contained 93.3% of the acute aluminum standard; 94.2% of the proposed copper criterion; 152.4% of the proposed lead criterion; and 105.7% of the proposed zinc criterion, expressed as total recoverable metal (Table 2). Thus, this test yielded acceptable results even though the copper concentration was substantially higher than the proposed site-specific criterion concentration.

Dissolved metal concentrations were well below corresponding proposed dissolved criteria for copper, lead, and zinc (Table 2). Further, the dissolved:total ratios observed in the mixture testing were much lower than that assumed in the EPA criteria documents or observed in single-metal WER testing in either laboratory or Chuitna water (Table 1). These lower ratios in mixture testing are not surprising given that the mixture contains aluminum and lead, two metals that readily combine with other metals, resulting in low dissolved metals in solution (e.g., aluminum or alum is a standard flocculant in wastewater treatment). This effect is even more pronounced in Chuitna site water, which contains a variety of complexing agents (e.g., organic carbon) and has a neutral pH (7.0). All the metals tested are relatively insoluble when combined at neutral pH. In fact, it is chemically not possible to manipulate the site water in a reasonable manner (i.e., without reducing the pH to very acidic and toxic levels) to achieve the dissolved criteria goals for these metals in combination.

Table 2. Summary of proposed metals criteria, tested concentrations, and survival of *Daphnia magna* test species in acute exposures.

			Total recoverable acute criterion (mg/L)	Measured total recoverable (mg/L)	Measured value as % of total criterion	Dissolved acute criterion (mg/L)	Measured dissolved (mg/L)	Measured value as % of dissolved criterion
Sample	Mean Survival	Metal						
Lab Control	95%	Al	0.7500	0.0050	0.7%	NA	0.0100	NA
		Cu	0.0377	0.0041	10.7%	0.0362	0.0005	1.4%
		Pb	0.2115	0.0005	0.2%	0.1972	0.0005	0.3%
		Zn	0.0618	0.0025	4.0%	0.0604	0.0025	4.1%
Site	100%	Al	0.7500	0.2325	31.0%	NA	0.0490	NA
		Cu	0.0377	0.0008	2.1%	0.0362	0.0015	4.1%
		Pb	0.2115	0.0005	0.2%	0.1972	0.0067	3.4%
		Zn	0.0618	0.0051	8.3%	0.0604	0.0068	11.3%
Spiked Site	85%	Al	0.7500	0.7000	93.3%	NA	0.1228	NA
		Cu	0.0377	0.0355	94.2%	0.0362	0.0145	40.1%
		Pb	0.2115	0.3225	152.4%	0.1972	0.0235	11.9%
		Zn	0.0618	0.0653	105.7%	0.0604	0.0308	50.9%

Results of the *P. promelas* mixture testing indicate that none of the three treatments resulted in significant ($\alpha = 0.05$) mortality (Table 3). The spiked site water sample contained 88.3% of the acute aluminum standard; 100.2% of the proposed copper criterion; 159.9% of the proposed lead criterion; and 109.7% of the proposed zinc criterion expressed as total recoverable (Table 3). Thus, this test yielded acceptable results even with substantially higher lead concentrations than its respective proposed criterion concentration.

Similar to the *D. magna* mixture test discussed above, dissolved metal concentrations were well below the corresponding proposed dissolved criteria for copper, lead, and zinc (Table 3) but very consistent with results observed using *P. promelas* (Table 2). Again, natural complexing agents and neutral pH of the Chuitna site water, in addition to the combination of metals being tested, make it impossible to achieve the dissolved metal concentration goals using approved toxicity testing protocols.

Table 3. Summary of proposed metals criteria, tested concentrations, and survival of *Pimephales promelas* (fathead minnow) test species in acute exposures.

Fingerprints of metals (radicals minus 7) test species in acute exposures								
Sample	Mean Survival	Metal	Total recoverable acute criterion (mg/L)	Measured total recoverable (mg/L)	Measured value as % of total criterion	Dissolved acute criterion (mg/L)	Measured dissolved (mg/L)	Measured value as % of dissolved criterion
Lab Control	100.0%	Al	0.7500	0.0050	0.7%	NA	0.0050	NA
		Cu	0.0377	0.0008	2.0%	0.0362	0.0005	1.4%
		Pb	0.2115	0.0005	0.2%	0.1972	0.0005	0.3%
		Zn	0.0618	0.0025	4.0%	0.0604	0.0043	7.0%
Site	97.5%	Al	0.7500	0.3200	42.7%	NA	0.0460	NA
		Cu	0.0377	0.0005	1.3%	0.0362	0.0005	1.4%
		Pb	0.2115	0.0005	0.2%	0.1972	0.0035	1.8%
		Zn	0.0618	0.0032	5.2%	0.0604	0.0045	7.4%
Spiked Site	97.5%	Al	0.7500	0.6625	88.3%	NA	0.1180	NA
		Cu	0.0377	0.0378	100.2%	0.0362	0.0158	43.5%
		Pb	0.2115	0.3375	159.5%	0.1972	0.0303	15.3%
		Zn	0.0618	0.0678	109.7%	0.0604	0.0348	57.5%

We believe that the spiked samples tested with both *D. magna* and *P. promelas* are accurate representations of a “worst-case” scenario that evaluates all proposed site-specific metal criteria at once. Highly ionic metal salts were used to spike metals in the mixture tests and chemical analyses confirmed that in the absence of ligands and other complexing agents (such as in Tetra Tech’s lab water), these metals occur in a highly soluble, dissolved state that is the most toxic form to aquatic life (USEPA 1994). Despite using total metal concentrations that at least met, and in some case exceeded, metal concentrations equivalent to proposed site-specific criteria, no significant effects on organism survival were observed in mixture testing. While dissolved concentrations of metals were much lower in testing, due to the chemical reactions that take place among the metals and with natural ligands in the site water mixture, this reflects a natural phenomenon that would occur in the Chuitna if free ionic, dissolved metals were in fact discharged to the stream. As noted previously, it is highly unlikely that the metals evaluated in this study would occur in the free ionic form because this form is highly unstable under natural conditions. As a result, dissolved copper, lead, and zinc would actually occur at even lower concentrations in the Chuitna than observed in this study.

Knowing the total and corresponding dissolved concentrations for each metal in site water mixture testing (Tables 2 and 3), it is possible to predict the total concentration of each metal that would theoretically be needed to achieve dissolved metal concentrations equivalent to the

proposed site-specific criteria, which are based on dissolved metal WERs. Table 4 summarizes these results. As can be seen, total copper and zinc concentrations would need to be more than doubled the proposed total criterion while lead would theoretically need to be more than 12 times the proposed total lead criterion. The concentration of lead needed is insoluble in water at neutral pH (> 1 mg/L lead is general insoluble at pH of 7.0 and 20°C). Thus, it is probably physically impossible to achieve the dissolved lead criterion concentration when mixed with the other metals in Chuitna surface waters, indicating that the results of mixture testing are conservative. Because these solutions were shown to be non-toxic to both test species used in generating these WER values, these results demonstrate that the proposed criteria are suitable for application to this system.

Table 4. Predicted total recoverable concentrations of each metal that would theoretically be needed to achieve proposed site-specific criteria concentrations based on dissolved metal water effect ratios determined by Tetra Tech in previous testing.

Metal	Proposed Acute Site-Specific Criterion (SSC) (mg/L)	Dissolved:Total Ratio (DTR)	Predicted Total Metal Concentration Needed (SSC/DTR) mg/L
Copper	0.036	0.42 ⁺	0.086
Lead	0.197	0.08 ⁺	2.462
Zinc	0.060	0.50 ⁺	0.120

⁺ From Table 1.

^{*} Derived from total concentrations reported in Tables 2 and 3.

ATTACHMENT A

Test Conditions for Confirmatory Metal Mixture Testing

Confirmatory Test Conditions

Species:	<i>Daphnia magna</i> and <i>Pimephales Promelas</i>
Replication:	4 replicates per treatment
Individuals/Rep:	10 (for both <i>P. promelas</i> and <i>Daphnia</i> tests)
Treatments:	Three total: Spiked Chuitna site water, unspiked Chuitna site water, and Control.
Control water:	Soft reconstituted. Normal (neutral) pH.
Metals Measured:	Aluminum, Copper, Lead, and Zinc. Total recoverable metals measured at initiation and test end; dissolved metals measured at test initiation, renewal at 24 hours, and at test end
Other Water Quality:	Initial sample: total and dissolved organic carbon and both total and dissolved metals (Aluminum, Copper, Lead, and Zinc). Test samples: pH, conductivity, dissolved oxygen, alkalinity, and hardness.